ELSEVIER

Biochimica et Biophysica Acta 1502 (2000) 264–272

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.elsevier.com/locate/bba

Pharmacokinetics of chronically administered all-*trans*-retinoyl-β-glucuronide in mice

Neil Sidell [a,*], Sayan Sawatsri [a], Michael J. Connor [b], Arun B. Barua [c], James A. Olson [c], Randal K. Wada [d]

[a] *Division of Research, Department of Gynecology and Obstetrics, Emory University School of Medicine, 4303 Woodruff Memorial Research Building, 1639 Pierce Drive, Atlanta, GA 30322, USA*

[b] *DTPC Inc., 4067 Mission Inn Ave., Riverside, CA 92501, USA*

[c] *Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011, USA*

[d] *Cancer Research Center of Hawaii and the Kapiolani Health Research Institute, Honolulu, HI 96813, USA*

Received 28 December 1999; received in revised form 25 April 2000; accepted 16 May 2000

Abstract

After the subcutaneous injection of retinoyl β-glucuronide (RAG), both RAG and retinoic acid (RA), formed by the hydrolysis of RAG in vivo, achieved peak plasma concentrations within 1–2 h. Thereafter, RA was rapidly cleared from the plasma whereas RAG was eliminated much more slowly. No significant changes were noted in the peak (2 h) plasma levels of RAG for treatment periods up to 56 days (one injection of RAG/day), in the clearance rate of RAG from plasma, or in plasma retinol concentrations. Similarly, no consistent decrease in plasma levels of the RA hydrolysis product was observed. Mice undergoing these long-term chronic treatments with RAG did not show any clinical manifestations of retinoid toxicity. Taken together, our findings that chronic dosing with RAG produces sustained levels of both the parent compound and the RA hydrolysis product, combined with the apparent low toxicity of RAG, suggest that RAG could be a safe and useful alternative to some retinoids which are presently being utilized in the clinic.

Keywords: Retinoid; Retinoic acid; Retinoyl glucuronide; Pharmacokinetics; Mouse

1. Introduction

Since the demonstration that all-*trans*-retinoic acid (RA) was a highly efficient compound for inducing remission in patients with acute promyelocytic leukemia [1], there has been considerable interest in the use of retinoids for other therapeutic applications in oncology, including Kaposi sarcoma [2], neuroblastoma [3], multiple myeloma [4], and head-and-neck tumors [5]. However, two limitations in the use of RA in the clinic are: (1) that this retinoid rapidly induces its own metabolism during chronic treatment by increased oxidation via the cytochrome P450 (CYP) enzyme system [6], and (2) its inherent toxicity. Because of the induced metabolism of RA, plasma RA concentrations are markedly reduced to almost undetectable levels within days of the initiation of drug administration. As a result, co-administration of CYP inhibitors such as ketoconazole and liarozole have been used to increase the plasma con-

* Corresponding author. Fax: +1-404-727-8615; E-mail: nsidell@emory.edu

0925-4439/00/$ – see front matter
PII: S0925-4439(00)00052-1

centrations of RA during long-term treatment, or drug 'holidays' have been utilized to allow for return of plasma drug clearance toward baseline levels [7,8]. This pharmacologic property of RA, its markedly increased metabolism with chronic dosing, has contributed to the limited use of this retinoid in oncologic applications. Indeed, there is now evidence that relapse from RA therapy of acute promyelocytic leukemia patients may, under certain circumstances, be due to a pharmacological inability to present a sustained effective drug concentration to the leukemic cells [9].

The glucuronide conjugate of retinoic acid (retinoyl β-glucuronide; RAG) has been the subject of intense interest because of its biological effectiveness in a variety of retinoid-sensitive systems, including the induction of cellular differentiation of certain tumor cell types [10,11]. At the same time, RAG demonstrates markedly reduced cytotoxicity and teratogenicity when compared to the RA parent compound [10]. Pharmacologic studies of RAG have indicated that its rate of clearance was relatively slow in well nourished animals, such that high plasma levels can be sustained for up to 12 h after the administration of a single subcutaneous or intravenous dose [12]. This kinetic behavior is in contrast to the rapid clearance observed with RA, which is no longer detectable in plasma within hours following administration.

In order to assess the possible use of RAG in long-term clinical applications, we have investigated both its conversion to RA and possible changes in its pharmacokinetics during chronic administration. We now demonstrate that unlike RA, daily administration of RAG for up to 2 months does not reduce peak plasma drug concentrations nor significantly change plasma retinol concentrations. Our data indicate that RAG can be hydrolyzed in vivo to RA and, furthermore, that daily RAG treatment results in peak RA plasma levels that do not decrease during the course of long-term treatment.

2. Materials and methods

2.1. Laboratory precautions

All work with retinoids or with plasma from RAG-treated mice was performed under dim light to reduce photodegradation.

2.2. Chemicals and solvents

All-*trans*-retinoyl β-glucuronide (RAG) was synthesized according to Becker et al. [13]. The purity of RAG was determined by HPLC [14]. Methanol, potassium acetate, acetic acid, butylated hydroxytoluene, anhydrous sodium sulfate, methylene chloride, and ethyl acetate were purchased from Fisher Scientific Co. (Fair Lawn, NJ). All-*trans*-RA, retinol, and retinyl palmitate for use as standards for HPLC were obtained from Sigma Chemical Co. (St. Louis, MO).

2.3. HPLC

The Emory Gynecology and Obstetrics HPLC setup is a Knauer variable wavelength monitor connected to a Rainin HPLC system, consisting of two Rabbit HP pumps, a pressure monitor, a mixing chamber, and a Rheodyne sample injector fitted with a 2 ml sample loop. The system is controlled from a Macintosh IIci using a Dynamax HPLC Method Manager. Retinoids were separated on a Varian (Rainin) 5 μm C_{18} column by reversed-phase HPLC procedure using a solvent flow rate of 1 ml/min as previously described [14]. For RAG and RA determination, the column was eluted isocratically with a solvent consisting of 13% 0.01 M potassium acetate–87% methanol adjusted to pH 5.6 with a few drops of acetic acid. For liver retinoids, the column was eluted isocratically with methanol at a flow rate of 2 ml/min.

2.4. Mice

Harland ICR female mice were housed and maintained in accordance with NIH guidelines for animal use and care under the supervision of Laboratory Animal Resources, Emory University. They received a standard pellet diet (Purina, St. Louis, MO) and tap water ad libitum. At 10–12 weeks of age, mice received either a single subcutaneous (s.c.) injection of RAG at the indicated concentrations per kg body weight (b.wt.) in the upper dorsum or daily s.c. injections for the indicated time periods in the long-term studies. RAG was prepared in 10% dimethyl-

sulfoxide–90% phosphate-buffered saline at stock concentrations such that the dosing volume was always 0.1 ml. Control mice were administered the vehicle only or were left untreated. Stock solutions of RAG were prepared every 2 weeks, aliquoted and frozen at −80°C, for daily use during the long-term chronic studies.

At the indicated time after RAG administration, mice were anesthetized using Metofane (methoxyflurane, Mallinckrodt Veterinary, Mundelein, IL). They were then exsanguinated until they expired by cardiac puncture into heparinized syringes. The blood was centrifuged for 12 min at $1200 \times g$ at room temperature and the plasma was recovered and stored at −20°C until analyzed by HPLC.

2.5. Hepatic vitamin A

Retinoids were extracted from liver by grinding tissue samples in methylene chloride in the presence of anhydrous sodium sulfate as previously described [15]. Briefly, a small amount (100–200 mg) of liver was added to a round-bottomed Pyrex glass tube that contained about 200 mg of anhydrous sodium sulfate. Methylene chloride (1 ml) was added and the sample homogenized using a mechanical tissue grinder. The sample was clarified by centrifugation (1500 rpm for 5 min). After the supernatant was recovered, four further extractions were conducted with ethyl acetate (1 ml), the supernatants sequentially pooled, and the pool evaporated to dryness under nitrogen. Just prior to chromatography, the residue was resuspended in 0.5 ml ethyl acetate.

2.6. Cell culture

The LA-N-5 human neuroblastoma cell line was grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, HEPES buffer and 50 IU/ml penicillin/streptomycin and 1 μg amphotericin (complete medium), as previously described [16]. For treatment of cells in culture, RAG was first dissolved in dimethylsulfoxide to a concentration of 5×10^{-2} mmol and then diluted in complete medium to the indicated concentrations. The effects of RAG on LA-N-5 cell proliferation was assessed by staining of cell protein with sulforhodamine (SRB) as described [17].

3. Results

3.1. In vivo kinetics

The mean concentrations of RAG and all-*trans*-RA found in plasma at various times during a 24 h period after RAG administration (30 μmol/kg b.wt.) are shown in Fig. 1. Both RAG and RA, resulting from the hydrolysis of RAG, reached peak plasma concentrations of 3.2 μM and 0.27 μM, respectively, 1–2 h after administration. As shown, the RA was rapidly cleared and was no longer detectable in the plasma at the 4 h time point. In contrast, the elimination of RAG was much slower, still showing micromolar concentrations 8 h after the s.c. injection. Twenty-four hours after administration, the plasma RAG concentration had decreased close to its baseline value. At selected time points, the presence of RAG was confirmed by treating the serum with β-glucuronidase, which then yielded RA. No attempts were made to distinguish between *trans* and *cis* isomers of the retinoids.

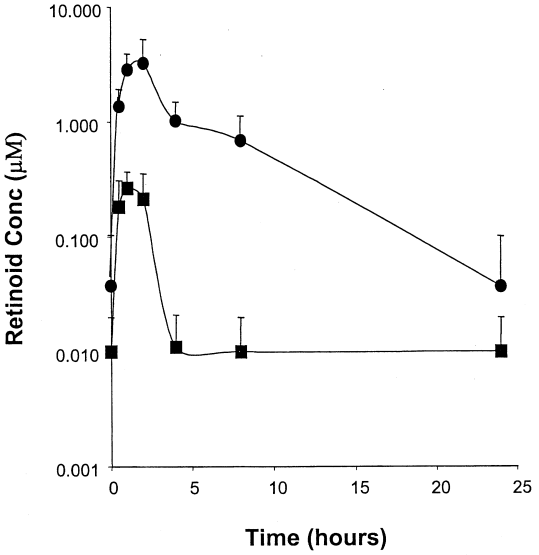

Fig. 1. Plasma concentration of RAG (●) and RA (■) following a single administration of RAG. Mice were injected s.c. with 30 μmol/kg b.wt. of RAG, then bled by cardiac puncture at the indicated times following the injection. Data represent mean values ± S.D. of 3–5 mice at each time point.

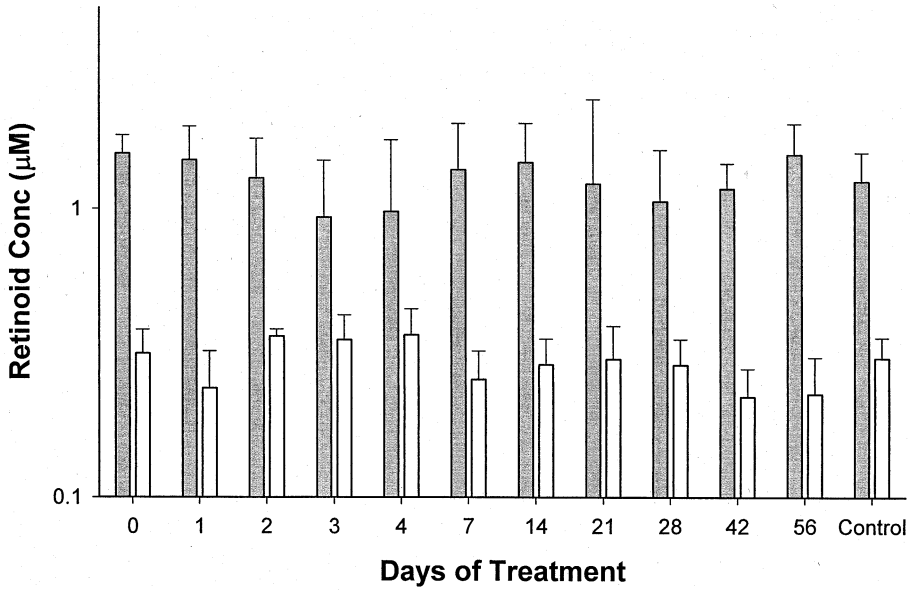

Fig. 2. Plasma concentration of RAG (gray bar) and retinol (ROL, open bar) during long-term, chronic administration of RAG. Mice were injected s.c. with 15 μmol/kg b.wt. of RAG once per day for up to 56 days. Blood was obtained by cardiac puncture 2 h following injection of RAG on the indicated day of treatment. Data represent mean values ± S.D. of 3–5 mice at each time point.

3.2. Chronic administration

All-*trans*-RAG was administered s.c. to mice at half the dose (15 μmol/kg b.wt.) given for the 24 h kinetic study above. The mice were injected once a day for up to 56 days. Plasma was obtained by cardiac puncture (3–5 mice/group) 2 h after the s.c. injections, which represented the time to peak plasma concentration of RAG and RA as determined from the 24 h kinetic study. Groups of mice were bled on the following time schedule representing the number of days from the initial RAG administration: 0, 1, 2, 3, 4, 7, 14, 21, 28, 42, 56. On days 0, 7, 14, 28, and 56 other groups of mice were also bled 4 h after the s.c. RAG injections in order to assess whether a shift in the kinetics of retinoid clearance occurred over the course of treatment. For additional control purposes, two groups (3 mice/group) of control mice were injected with RAG along with the 56-day-treated groups, and bled 2 and 4 h after injections. Fig. 2 demonstrates mean RAG concentrations (± S.D.) of approximately 1.28 ± 0.22 μM were achieved at 2 h throughout the 56 day treatment, whereas the mean 4 h plasma levels of RAG were 0.54 ± 0.13 μM (not shown). The peak (2 h) plasma levels of RAG at all times throughout the 56 day treatment period were similar. Likewise, the plasma concentration of RAG in the 4 h samples were consistently 30–50% of those at 2 h at all times during this period. Retinol levels did not change significantly during the course of this chronic RAG treatment.

In contrast to our finding that RA could be readily found in plasma following the higher RAG dose given in the short-term 24 h kinetic analysis (Fig. 1), RA was not consistently detected in the plasma sam-

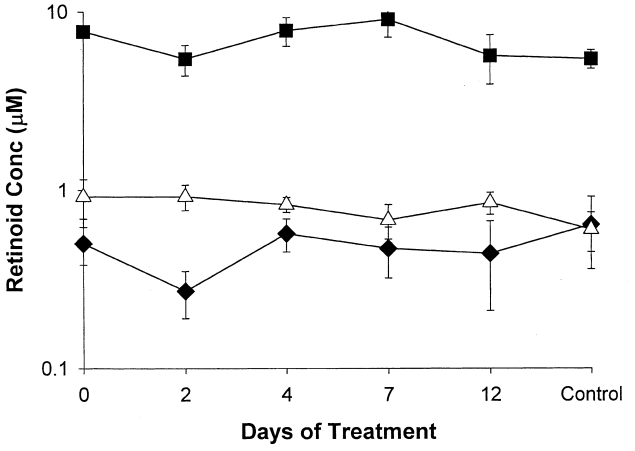

Fig. 3. Plasma concentrations of RAG (■), RA (◆), and retinol (ROL, △) at 2 h after the daily subcutaneous treatment of mice with RAG (30 μmol/kg b.wt.) for up to 12 days. Values represent mean ± S.D. of 3–5 mice at each time point.

ples from this 56 day study. Some samples, however, showed RA levels near the limit of detection of our HPLC system (~0.05 μM). There was no time-related pattern as to which plasma specimens showed low levels of RA such that positive samples were obtained from various mice at both the beginning and end of the 56 day treatment. Therefore, in a third treatment protocol, we injected RAG s.c. at the higher dose (30 μmol/kg b.wt.) once each day for up to 12 days. Mice were then bled 2 h after the RAG injections on days 0, 2, 4, 7, and 12. As before, the mice on the last day (day 12) included a group that had been treated continuously with RAG for 12 days and a control group that was treated with RAG for the first time on day 12. Fig. 3 shows that mean plasma levels of RAG at 2 h ranged between 5.5 and 9.0 μM and that these peak levels were not different throughout the 12 days of treatment. In these studies, all-*trans*-RA was detected in all plasma samples and ranged between 0.27 and 0.64 μM. These 2 h plasma levels of RA did not decrease throughout the 12 day treatment or between the 12-day-treated mice and controls. As before, the mean endogenous plasma level of retinol (0.80 ± 0.13 μM) was not altered during the course of this RAG treatment protocol.

3.3. Weight gain

In the 56 day chronic administration of RAG, there were no significant differences in initial or terminal weights, water consumption, social behavior, or apparent vitality between RAG-treated and control groups. Mean weights (± S.D.) after 16 days of treatment were 28.90 ± 1.15 g for controls versus 28.38 ± 1.84 g for RAG-treated ($P = 0.614$) and after 56 days were 31.7 ± 1.42 g for controls versus 30.55 ± 0.52 g for RAG-treated ($P = 0.12$) (Fig. 4). There were no observable clinical manifestations of retinoid toxicity in the RAG-treated group, as has been reported in mice when chronically treated with other retinoids (e.g. weight loss, alopecia, and scaly skin [18].

3.4. Liver vitamin A levels

The mean (± S.D.) vitamin A concentration of the 56 day control group (4.79 ± 0.49 μmol/g tissue) was

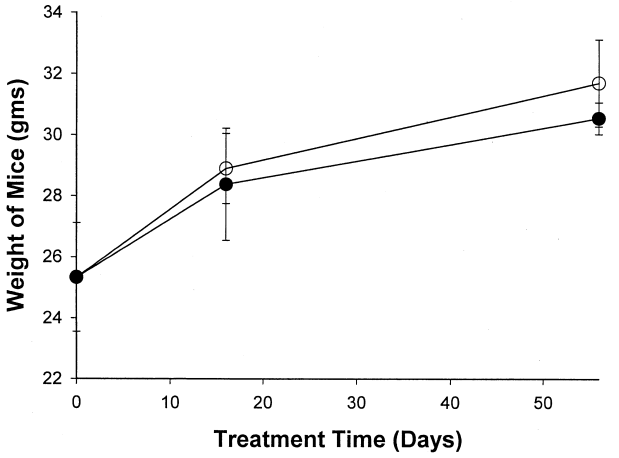

Fig. 4. Lack of effect of long-term RAG treatment on weight gain of mice. Twelve-week-old mice were chronically injected with RAG (●) as described in Fig. 2 and their weights compared with control mice (○) on the 16th and 56th day of treatment. Data represent mean ± S.D. of four mice in each group.

significantly higher ($P < 0.005$) than that of the RAG-treated mice (3.66 ± 0.51 μmol/g). There were no significance differences in liver weights between these two groups of mice (not shown). In both control and RAG-treated mice, greater than 98% of hepatic vitamin A was stored in the form of retinyl esters.

3.5. Biologic activity of RAG on human tumor cells

Our in vivo studies indicated that micromolar blood levels of RAG can be maintained during chronic long-term administration. To determine whether such levels could have biological significance, we assessed the ability of RAG to inhibit the growth and induce morphologic differentiation of the LA-N-5 human neuroblastoma cell line. This cell type has been shown to be exquisitely sensitive to differentiation induction by retinoids, and neuroblastoma is now treated with retinoids in the clinic [3]. As shown in Fig. 5, RAG induced neurite outgrowth from LA-N-5 cells. This effect first became apparent after about 2 days of continuous exposure in culture, with maximal increases in the formation of neurites occurring at RAG concentrations in the 5–10 μM range after extended periods (> 6 days). Concentrations below 0.1 μM produced no noticeable morphologic effects. No decrease in the percentage of viable cells was detected in treated cultures as compared

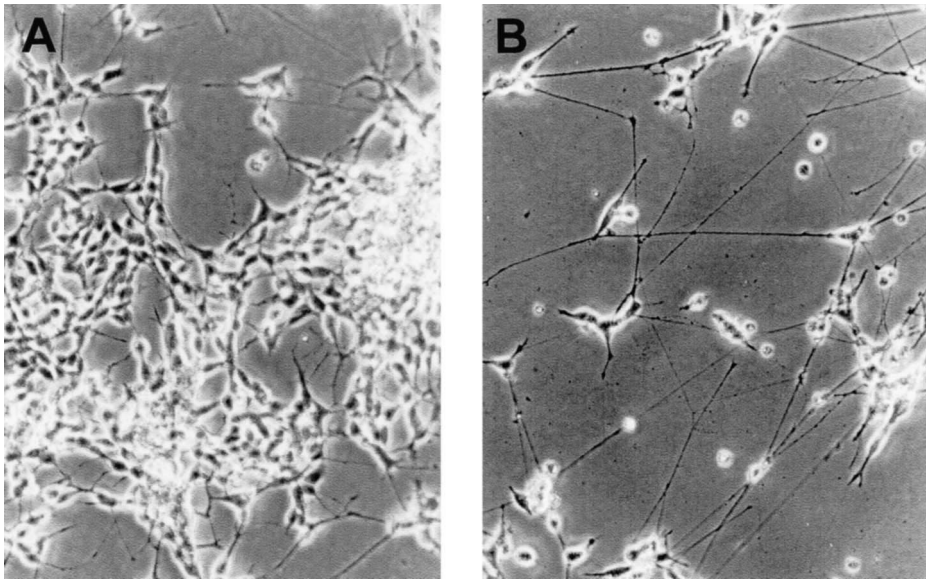

Fig. 5. Morphology of LA-N-5 neuroblastoma cells in the absence and presence of RAG. Cultures were treated for 10 days with vehicle (A) or 5 μM RAG (B). Photographed under phase contrast. ×150.

with control cultures. These morphologic changes are similar to those extensively reported after RA treatment of LA-N-5 [19], with the exception that maximal neurite outgrowth is seen with RA at somewhat lower concentrations (>1 μM).

Fig. 6 shows the dose-dependent effects of RAG on the growth of LA-N-5 cells as assessed by SRB protein staining. As seen, greater than 40% growth inhibition was achieved at RAG concentrations above 1 μM in these 5 day cultures, while little effect was found below 10^{-8} M. For comparison, the growth inhibitory effects of RA were also tested in parallel cultures. The dose–response curves indicate that RA was 5–10-fold more potent than RAG in inhibiting the growth of LA-N-5 cells. This greater potency of RA versus RAG was consistent with our morphologic observations of the relative ability of the two compounds to induce neurite outgrowth.

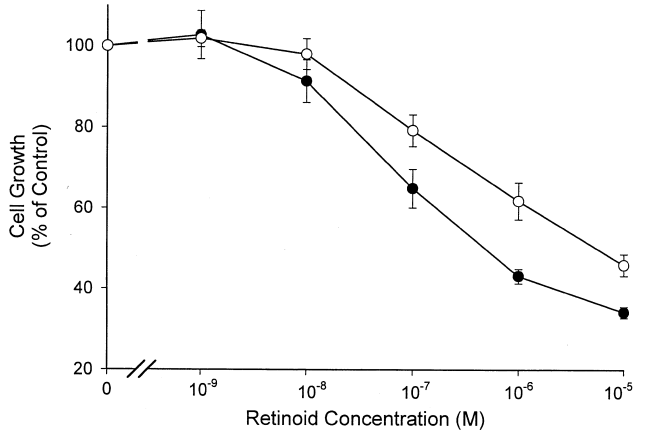

Fig. 6. Dose–response curves showing the effects of RAG (○) and RA (●) on LA-N-5 cell growth after 5 days of culture. Values represent the mean ± S.D. of triplicate samples of a typical experiment and are expressed as percent of control cultures as assessed by the SRB protein assay.

4. Discussion

Nau et al. [12] reported that a single s.c. injection of RAG (20 μmol/kg b.wt.) into pregnant NMRI female mice gave peak plasma concentrations at 2 h of RAG (2.0 μM) and of RA (0.7 μM). Thereafter, the RA concentration decreased rapidly to 0.05 μM at 4 h, whereas the RAG concentration fell much more slowly, e.g., 1.2 μM at 4 h and 0.40 μM at 8 h. Interestingly, when the same single dose of RA was injected s.c. into NMRI mice, the peak plasma concentration of RA at 1 h was only 0.1 μM, followed by a rapid decline.

When single s.c. injections of RAG were administered to Harland ICR female mice in our study, the results were qualitatively similar to those of Nau et al. [12], but quantitatively somewhat different. Both RAG and its hydrolysis product RA peaked at 1–2 h

followed by a relatively slow decrease in the RAG concentration and a rapid fall in the RA concentration. The peak RAG concentration after a single dose (30 μmol/kg b.wt.) in our studies, however, was approximately 1.5-fold higher than that in Nau's work, whereas the peak RA concentration was lower (~0.3 μM). Thus, the ratio of peak RA/peak RAG was 0.08 in our study and 0.35 in Nau's. This difference may reflect a more rapid hydrolysis rate of RAG to RA in NMRI mice than in ICR mice and/or differences in the clearance rate of RA between strains. Regarding the latter possibility, 0.05 μM RA could be detected in the plasma of NMRI mice 4 h after RAG injection [12] while essentially no RA (≤0.01 μM) was detected in our study at the same time point in ICR mice. Thus, the rate of conversion of RAG to RA and possibly the rate of RA clearance seem to be strain-dependent. In Sprague–Dawley rats, the ratio of peak RA/peak RAG after a single s.c. injection of RAG (20 μmol/kg b.wt.) is 0.07, similar to that in ICR mice (A. Ueltschy and J.A. Olson, unpublished data). Similarly, in our 12 day experiment, the mean ratio of peak RA/peak RAG at 2 h was found to be approximately 0.06 (Fig. 3) throughout the treatment period, suggesting that the rate of hydrolysis of RAG to RA and the latter's clearance remained relatively constant during this period.

In pregnant NMRI mice, Nau et al. [12] found that a single s.c. dose of RAG (20 μmol/kg b.wt.) given on gestational day 11 was more teratogenic than the same s.c. dose of RA. In contrast, RA, given orally or by s.c. injection on gestational day 11, is much more teratogenic than RAG in Sprague–Dawley rats ([20], A. Ueltschy and J.A. Olson, unpublished observations). Although the relative teratogenicity of RA and RAG has not been examined in Harland ICR mice, daily s.c. dosing with similar amounts of RAG (15 μmol and 30 μmol/kg b.wt.) for up to 56 days caused no toxic manifestations in our study. NMRI mice thus seem to be much more sensitive to both RA and RAG than other mice or rats that have been studied.

Although blood plasma levels of RAG and its conversion to RA following RAG administration have been studied in a variety of species under both vitamin A-deficient and -sufficient conditions [12,13,20], the present work has for the first time tested the consequences of long-term RAG treatment on resulting retinoid plasma levels. Importantly, daily s.c. injections of RAG resulted in peak plasma levels that did *not* decrease during the course of a 2 month treatment period. Thus, multiple RAG treatment does not appear to induce its own metabolism, as is the case for all-*trans*-RA and, to a lesser extent, for 9-*cis*-RA [21]. Furthermore, when RAG was given at daily doses (30 μmol/kg b.wt.) for 12 days, which consistently resulted in peak plasma RA concentrations of about 0.5 μM, we detected no significant changes in RA concentrations over the 12 day period. Thus, the production of RA from the continuous hydrolysis of persistent RAG levels apparently does not result in the induction of CYP oxidative enzymes [6,22] during at least the first 12 days. The RA levels at 2 h in the 56 day study at a lower daily dose of RAG (15 μmol/kg b.wt.), however, were less consistent such that further conclusions cannot be drawn at this time.

The biological relevance of the sustained plasma levels of RAG achieved in our study was investigated using the retinoid-responsive LA-N-5 human neuroblastoma cell line. Previous work by us [19] and others [23], has demonstrated that neuroblastoma cells, as a group, are remarkably sensitive to retinoid-induced differentiation both in vitro and in vivo [3,24]. Our results suggest that the doses and methodology of RAG administration utilized could show clinical activity against neuroblastoma due to the high (1–10 μM) blood levels of RAG that were obtained, and by the fact that RA, as a hydrolysis product of RAG, did not decrease during treatment. It is unknown whether or not RAG itself is biologically active in neuroblastoma or other cell types, or must be converted to RA or other active metabolites. In this regard, previous studies have demonstrated that RAG does not bind to RA nuclear receptors [25], suggesting that metabolic conversion may be required.

Plasma retinol levels did not change appreciably during RAG administration. Although dietary or supplemental RA can lower plasma levels of retinol [26–30], the amounts of RA produced from RAG in our study were apparently insufficient to induce this effect. RA added to the diet of rats has also been shown to enhance vitamin A reserves in their livers [26,28], probably by sparing the need for vitamin A

in peripheral tissues. The uptake of retinyl ester-containing chylomicra by the liver and the release of a retinol–retinol binding protein complex from the liver seemed normal in RA-treated rats [26]. Thus, it surprised us that long-term RAG treatment significantly reduced ($P < 0.005$) liver reserves of vitamin A from 4.79 ± 0.49 μmol/g to 3.66 ± 0.51 μmol/g in our current study. A possible explanation is that RAG is competing with RA at regulatory sites for vitamin A metabolism. Thus, RAG might inhibit the stimulatory effect of RA on vitamin A uptake by intestinal cells [31] or might offset the sparing effect of RA on liver reserves of vitamin A [26,28]. Clearly, the effect of RAG on liver reserves of vitamin A merits further attention.

In conclusion, the observation that chronic subcutaneous dosing with RAG results in sustained elevated levels of this compound may have important therapeutic implications in clinical medicine. Previous work and our present studies have shown that RAG can support the growth of vitamin A-deficient mice, induce the differentiation of acute promyelocytic leukemia and neuroblastoma cells, and inhibit the development of prolactin-induced mammary glands [10,32]. Thus, the fact that levels of RAG in the micromolar range can be maintained for long periods without apparent adverse side effects may well serve as an alternative to intermittent applications of RA. Furthermore, the fact that long-term daily administration of RAG can result in chronically elevated plasma levels of RA suggests a more effective method for delivering RA itself to selective sites or cell types.

Acknowledgements

This work was supported by NIH Grants CA43503 (N.S.), DK39733 (J.A.O.), NS34432 (R.K.W.), and a generous Pramongkutklao Foundation grant from the Pramongkutklao Hospital, Bangkok, Thailand (S.S.).

References

[1] S. Castaigne, C. Chomienne, M.T. Daniel, P. Ballerini, R. Berger, P. Fenaux, L. Degos, All-*trans*-retinoic acid as differentiation therapy for acute promyelocytic leukemia, Blood 76 (1990) 1704–1709.

[2] P.S. Gill, B.M. Espina, T. Moudgil, S. Kidane, J.A. Esplin, A. Tulpule, A.M. Levine, All-*trans* retinoic acid for the treatment of AIDS-related Kaposi's sarcoma: results of a pilot phase II study, Leukemia 8 (1994) 26–32.

[3] K.K. Matthay, J.G. Villablanca, R.C. Seeger, D.O. Stram, R.E. Harris, N.K. Ramsay, P. Swift, H. Shimada, C.T. Black, G.M. Brodeur, R.B. Gerbing, C.P. Reynolds, Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-*cis*-retinoic acid, New Engl. J. Med. 341 (1999) 1165–1173.

[4] N. Swaminathan, G. Lopez-Berestein, S. Rudikoff, Assessment of all-*trans* retinoic acid (ATRA) efficacy as a single agent in primary lymphoid neoplasia, Med. Oncol. 16 (1999) 119–128.

[5] N. Voravud, S.M. Lippman, R.S. Weber, G.I. Rodriquez, D. Yee, I.W. Dimery, C.L. Earley, D.D. Von Hoff, W.K. Hong, Phase II trial of 13-*cis*-retinoic acid plus interferon-alpha in recurrent head and neck cancer, Invest. New Drugs 11 (1993) 57–60.

[6] M.B. Regazzi, I. Iacona, C. Gervasutti, M. Lazzarino, S. Toma, Clinical pharmacokinetics of tretinoin, Drug Dispos. 32 (1997) 382–402.

[7] J.P. Van Wauwe, M.C. Coene, J. Goossens, G. Van Nijen, W. Cools, W. Lauwers, Ketoconazole inhibits the in vitro and in vivo metabolism of all-*trans*-retinoic acid, J. Pharmacol. Exp. Ther. 245 (1988) 718–722.

[8] P.C. Adamson, J. Bailey, J. Pluda, D.G. Poplack, S. Bauza, R.F. Murphy, R. Yarchoan, F.M. Balis, Pharmacokinetics of all-*trans*-retinoic acid administered on an intermittent schedule, J. Clin. Oncol. 13 (1995) 1238–1241.

[9] M. Cornic, L. Delva, S. Castaigne, P. Lefebvre, N. Balitrand, L. Degos, C. Chomienne, In vitro all-*trans* retinoic acid (ATRA) sensitivity and cellular retinoic acid binding protein (CRABP) levels in relapse leukemic cells after remission induction by ATRA in acute promyelocytic leukemia, Leukemia 8 (1994) 914–917.

[10] A.B. Barua, Retinoyl β-glucuronide: A biologically active form of vitamin A, Nutr. Rev. 55 (1997) 259–267.

[11] D. Janick-Buckner, A.B. Barua, J.A. Olson, Induction of HL-60 cell differentiation by water-soluble and nitrogen-containing conjugates of retinoic acid and retinol, FASEB J. 5 (1991) 320–325.

[12] H. Nau, M.M.A. Elmazar, R. Ruhl, R. Thiel, J.O. Sass, All-*trans*-retinoyl-β-glucuronide is a potent teratogen in the mouse because of extensive metabolism to all-*trans*-retinoic acid, Teratology 54 (1996) 150–156.

[13] B. Becker, A.B. Barua, J.A. Olson, All-*trans*-retinoyl β-glucuronide: New procedure for chemical synthesis and its metabolism in vitamin A-deficient rats, Biochem. J. 314 (1996) 249–252.

[14] M.J. Connor, M.H. Smit, Retinoic acid formation from retinol in the skin, Biochem. Pharmacol. 36 (1987) 919–924.

[15] S.M. Puhvel, M.J. Connor, M. Sakamoto, Vitamin A defi-

ciency and the induction of cutaneous toxicity in murine skin by TCDD, Toxicol. Appl. Pharmacol. 107 (1991) 106–116.
[16] N. Sidell, C.A. Lucas, G.W. Kreutzberg, Regulation of acetylcholinesterase activity by retinoic acid in a human neuroblastoma cell line, Exp. Cell Res. 155 (1984) 305–309.
[17] H. Huang, S.C. Shu, J.H. Shih, C.J. Kuo, I.D. Chiu, Antimony trichloride induces DNA damage and apoptosis in mammalian cells, Toxicology 129 (1998) 113–123.
[18] C. Lindamood 3d, H.D. Giles, D.L. Hill, Preliminary toxicity profile of arotinoids SMR-2 and SMR-6 in male B6D2F1 mice, Fund. Appl. Toxicol. 8 (1987) 517–530.
[19] N. Sidell, A. Altman, M.R. Haussler, R.C. Seeger, Effects of retinoic acid (RA) on the growth and phenotypic expression of several human neuroblastoma cell lines, Exp. Cell Res. 148 (1983) 21–30.
[20] D.B. Gunning, A.B. Barua, J.A. Olson, Comparative teratogenicity and metabolism of all-*trans* retinoic acid, all-*trans* retinoyl β-glucose, and all-*trans* retinoyl β-glucuronide in pregnant Sprague-Dawley rats, Teratology 47 (1993) 29–36.
[21] S. Wadler, E.L. Schwartz, P. Anderson, C.D. Runowicz, L. Chuang, G. Del Priore, H. Hochster, G. Goldberg, A. Fields, G. Loewen, H. Haynes, Preliminary phase II clinical and pharmacokinetic study of 9-*cis* retinoic acid in advanced cervical cancer. New York Gynecologic Oncology Group, Cancer J. Sci. Am. 5 (1999) 165–170.
[22] S.S. Abu-Abed, B.R. Beckett, H. Chiba, J.V. Chithalen, G. Jones, D. Metzger, P. Chambon, M. Petkovich, Mouse P450RAI(CYP26) expression and retinoic acid-inducible retinoic acid metabolism in F9 cells are regulated by retinoic acid receptor gamma and retinoid X receptor alpha, J. Biol. Chem. 274 (1998) 2409–2415.
[23] C.P.F. Redfern, P.E. Lovat, A.J. Malcolm, A.D.J. Pearson, Differential effects of 9-*cis* and all-*trans* retinoic acid on the induction of retinoic acid receptor-beta and cellular retinoic acid-binding protein-II in human neuroblastoma cells, Biochem. J. 304 (1994) 147–154.
[24] E. Abemayor, B. Chang, N. Sidell, Effects of retinoic acid on the in vivo growth of human neuroblastoma cells, Cancer Lett. 55 (1990) 1–5.
[25] B.P. Sani, A.B. Barua, D.L. Hill, T.W. Shih, J.A. Olson, Retinoyl beta-glucuronide: lack of binding to receptor proteins as related to biological activity, Biochem. Pharmacol. 43 (1992) 919–922.
[26] B. Keilson, B.A. Underwood, J.D. Loerch, Effects of retinoic acid on the mobilization of vitamin A from the liver of rats, J. Nutr. 109 (1979) 787–795.
[27] C. Weber, E. Dumont, Pharmacokinetics and pharmacodynamics of 9-*cis*-retinoic acid in healthy men, J. Clin. Pharmacol. 37 (1997) 566–574.
[28] B. Periquet, A. Periquet, A. Bailly, J. Ghisolfi, J.P. Thouvenot, Effects of retinoic acid on hepatic cytochrome P-450 dependent enzymes in rats under different vitamin A status, Int. J. Vitam. Nutr. Res. 56 (1986) 223–229.
[29] L.E. Gerber, J.W. Erdman Jr., Comparative effects of all-*trans* and 13-*cis* retinoic acid administration on serum and liver lipids in rats, J. Nutr. 110 (1980) 343–351.
[30] A.B. Barua, P.K. Duitsman, D. Kostic, M. Barua, J.A. Olson, Reduction of serum retinol levels following a single oral dose of retinoic acid in humans, Int. J. Vitam. Nutr. Res. 67 (1997) 423–425.
[31] M.S. Levin, A.E. Davis, Retinoic acid increases cellular retinol binding protein II mRNA and retinol uptake in the human intestinal Caco-2 cell line, J. Nutr. 127 (1997) 13–17.
[32] R.B. Mehta, A.B. Barua, J.A. Olson, R.C. Moon, Effects of retinoid glucuronides on mammary gland development in organ culture, Oncology 48 (1991) 505–509.